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DIETARY FACTORS AFFECTING EXOGENOUS AND ENDOGENOUS SOURCES OF FAT AND CARBOHYDRATE FOR ENERGY PRODUCTION AND SYNTHESIS

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INTRODUCTION: OBJECTIVES AND THEIR APPLICATION TO THE SPACE MISSION

Several conditions peculiar to space travel complicate man's usual nutritional and dietary requirements: 1 - a lack of space for storage of food, 2 - possible elongation of flight time or delays in recovery necessitating periods of inanition, 3 - constant caloric demand to maintain acute mental awareness without complications of hypoglycemia and ketosis, as well as 4 - increased gravitational force during lift off and 5 - weightlessness during the actual flight.

We have been investigating the effect of diets that would provide calories endogenously and yet maintain an adequate concentration of circulating glucose to meet the requirements of the central nervous system (CNS). Tissues other than the CNS utilize either carbohydrate or fat to meet their energy demands. Fat being the most concentrated form of energy would be the energy source of choice for space travel. We have shown that fat stores can be built up within the rat by feeding diets high in either fat or carbohydrate (Previous Progress Report, July 1968), thereby providing a caloric store to be consumed when needed. The fatty acid content of these stores developed from each dietary regimen is not the same. We have shown that the fat of animals fed the high fat diet reflects the fatty acid content of the diet while the fat of those fed high carbohydrate contains the fatty acids that are characteristically synthesized by the animal.

Unfortunately, the common natural lipids are ketogenic and cannot provide glucose or glucose precursors to maintain normal CNS function. Because the stores of glucose, mainly in the form of glycogen, in the body are small, the animal relying on endogenous sources of energy must form glucose from

other compounds, mainly the glucogenic amino acids. The liver is the major site of this gluconeogenesis.

It has long been known that the previous nutritional state of the animal can influence lipogenesis and glucose utilization in adipose tissue (1 - 3) and liver (4) and, also, hepatic glucose formation (5 - 8). While these earlier studies were conducted after short term feeding periods, we have shown that the enzymatic profile of adipose tissue and liver is still different in animals fed different diets for a matter of months (Annual Progress Report, July, 1968).

These differences in enzymatic activity and body composition probably have grave implications on the ability of the animal to survive a stressful situation. For example, the feeding of a diet rich in available carbohydrate increases fatty acid synthesis in liver (4) and adipose tissue (1). Such dietary treatment also increases the activities of many hepatic enzymes concerned with glucose utilization (9) and decreases the activity of those concerned with gluconeogenesis (6, 7). These enzymatic changes could leave the animal at a disadvantage for coping with a subsequent fast. On the other hand, diets rich in protein that tend to increase the rate of gluconeogenesis by the liver (8) might provide a more favorable enzymatic complement with which to enter the fast.

The differences in body composition resulting from the previous diet could also be a factor in the response to nutritional stress. The metabolic products of tissue components utilized during the fasting state for example, would be quite different in animals of differing body composition. Those with a higher fat content might show a propensity for ketosis whereas those with less fat might tend to mobilize protein as well as fat, thereby simul-

taneously producing precursors of glucose and ketone bodies. Hence, these latter animals would be more capable of maintaining the physiologically required concentrations of blood glucose than their fatter counterparts.

Feeding a diet containing a fatty acid with an odd number of carbon atoms would provide a unique solution to the problem of supplying energy as well as glucogenic substances during fasting. Fat stores containing straight chain fatty acids with an odd number of carbons would be broken down to propionate, a ready precursor of glucose. In contrast, fatty acids with an even number of carbon atoms yield acetate, a ketogenic rather than a glucogenic compound.

Because diet plays such an important role in regulating the enzymatic activity of adipose tissue and liver as well as the body composition of the animal, we propose to study the effects of diet upon the metabolic response of rats to a nutritional stressor. We have chosen fasting as the first stress condition to investigate.

In view of the above comments, we have devised diets that would: 1 - favor endogenous formation of fatty acids, i.e., low in fat and high in carbohydrate; 2 - favor accumulation of dietary fat in adipose tissue and decrease endogenous fatty acid synthesis, i. e., high fat diets; 3 - favor a high rate of gluconeogenesis, i. e., high protein diets; or 4 - fill the fat stores with odd carbon-chain fatty acids. We are examining the following indicators of the metabolic response to each diet: 1 - fatty acid formation in both isolated hepatic and adipose tissue; 2 - glucose formation by liver slices; 3 - the activities of key enzymes concerned with a) glucose utilization, b) lipogenesis, and c) gluconeogenesis; 4 - composition and fatty acid content of the total carcass, liver and adipose

tissue; and 5 - the plasma concentration of free fatty acids* and glucose. After the rats have been fed these specially prepared diets for at least four weeks, a period which we have shown was sufficient to alter the body composition (Annual Progress Report, July, 1968), they are divided into two groups. The above-mentioned metabolic parameters of one group are examined at this time (control group) and those of the other group are measured after a prolonged fast (7 days).

The results of these studies will indicate whether the previous dietary history alters the metabolic response to stress, in this immediate case, inanition. More importantly, they will allow us to predict which diet should be tested in astronautical studies. The lack of sufficient utilization of body stores for the purpose of maintaining a positive energy balance could be a factor in many metabolic upsets, including those of mineral metabolism (11, 12), observed in inactive subjects.

PRELIMINARY RESULTS

The present study is a logical extension of work performed on this grant previously. Two of the diets used in that earlier study, S and M (Table I), are being fed currently. Hence, the composition and enzymatic activity of the tissues of the control rats can act, not only as fed counterparts for those fasted, but also provide additional data for the previous study. This additional information will increase the degree of confidence in the differences observed previously.

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*Ketone bodies are derived from free fatty acids. Free fatty acids are an accurate gauge of the ketogenic state (10).

The results, thus far, confirm the previous unique findings that enzymatic differences occur in rats fed two different diets for a long period. When compared to the activity of rats fed the high carbohydrate diet, the activity of hepatic glucose-6-P dehydrogenase, 6-P-gluconate dehydrogenase, citrate lyase and total hexokinase were lower in rats fed a diet containing 45% fat. When the activities of these enzymes were measured in adipose tissue similar differences were observed except that malic enzyme was much lower in the fat fed animals while the total hexokinase activity was the same for both dietary groups.

The enzymatic profile exhibited in the animals fed these two diets would indicate that feeding fat decreases the formation of fatty acids in both liver and adipose tissue. Such an interpretation is verified by the lower incorporation of acetate into fatty acids by the tissues of the fat fed rats than by those fed diet S (Present study and Previous Progress Report, July, 1968).

The high protein diet was added in the current series of experiments to complete the examination of diets each rich in one of the basic foodstuffs (Table I). The activity of the hepatic enzymes measured indicated little difference between the enzymatic response to this diet and that to the high carbohydrate diet. In contrast, the activity of enzymes related to fat synthesis, both dehydrogenases, citrate lyase, and malic enzyme, were much lower in the adipose tissue of the protein fed animals than the activity of the same enzymes in rats fed a diet rich in carbohydrate. Clearly, long term feeding of carbohydrate increases the lipogenic capacity of adipose tissue.

The differences in the activity of the gluconeogenetic enzymes being

measured in the present study, pyruvate carboxylase and pyruvate carboxykinase, are not sufficient to indicate definite trends. When more measurements have been made a pattern of long term dietary effects may develop.

The experiment with animals fed the three diets, each rich in a different basic foodstuff, for four weeks and then fasted for a week was conducted at the time of preparation of this report. Until all the analyses are complete, we cannot interpret the effect of these diets on subsequent fasting.

In addition to those diets mentioned above, another one, containing heptadecanoic acid as the sole source of fat (Table I), was studied. This straight chain fatty acid which contains 17 carbon atoms was added to the diet in the same concentration as the saturated fat used in diet M. It is of sufficient length to directly enter the lymphatic system (13 - 15), thereby, avoiding degradation in the liver. It is also one of the few fatty acids of its type commercially available. Because of its cost we have conducted only a pilot study of four days feeding and seven days fast, to verify the worth of this approach. The preliminary studies with this diet are now completed.

Feeding C_{17} fatty acid clearly changes the fatty acid composition of all tissues examined, even though it was fed for only four days (Table II). The changes in epididymal adipose tissue do not reflect the fatty acid composition of the diet as markedly as animals fed plastine for 4-6 weeks. However, liver does show a significant rise in its content of this unique acid. We believe these differences in the distribution of the fatty acid into fat stores of animals fed the M and C_{17} diets are partially due to

length of the feeding period and partly to the fact that the odd carbon chain fatty acid was fed as the fatty acid rather than as triglyceride. Future experiments are planned in which C_{17} will be fed for long periods of time as the triglyceride as well as the free acid.

At this time, the only results available with fasted animals are those with rats previously fed heptadecanoic acid. Therefore, we cannot, compare the metabolic response of these animals to those fed the other diets and then fasted. However, it is valid and worthwhile to compare the fasted ones with their counterparts fed heptadecanoic acid. The results indicate that, during fasting, fatty acid synthesis from acetate was decreased. The activity of many hepatic enzymes was decreased, especially malic enzyme, citrate lyase and total hexokinase (Table II). Such a response to fasting has been reported previously. In this case, the change in activity of malic enzyme and citrate lyase may be related to the decrease in lipogenesis. (8, 16).

It is interesting that adipose tissue did not parallel the response of liver. Metabolic activity of this tissue was actually greater after the fast. Oxidation of acetate and lipogenesis were both markedly increased by fasting. As a result of this type of dietary manipulation a marked increase in the two enzymes of the pentose phosphate cycle, (the source of TPNH for fatty acid synthesis) was also observed. In this tissue, however, the activities of malic enzyme and citrate lyase, two enzymes supposedly related to lipogenesis, were either lower or the same at the end of the fast than at the start. Thus, in contrast to the situation in liver, lipogenesis and the activities of malic enzyme and citrate lyase do not parallel each other.

At first glance, the response of adipose tissue in fasting rats prefed the diet containing C_{17} fatty acid would be disadvantageous to survival. Efficient utilization of the limited nutrients during fasting would seem to call for a shuting down of lipogenesis and oxidative reactions in adipose tissue rather than an increase. The changes observed may be related to the fact that the C_{17} diet was rich in fat, a situation inhibiting lipogenesis. Fasting may have released this inhibition to the detriment of the animal. The response of fasted rats previously fed the other diets will be followed with interest.

The activity of hepatic pyruvate carboxykinase was two-fold, higher in the fasted animals than in the C_{17} fed animals. Such a response is expected if this enzyme is rate determining in glucose formation. Indeed, glucose formation from alanine was increased in the livers of the fasted rats. The activity of pyruvic carboxylase remained unchanged. This surprising response indicates that under the conditions of our study,this enzyme while participating in the gluconeogenic process does not control the rate of glucose formation.

Fasting, of course, reduced the amount of fat in the animal, and also altered the fatty acid composition of both liver and adipose tissue (Table III).

The concentration of plasma glucose and free fatty acids in these C_{17} fed rats cannot be compared to normal values reported in the literature because these animals were on a high fat diet. Neither concentration was markedly altered by prolonged fasting (Table IV). While we cannot say how animals fed the other diets will respond, it appears that stores of fat containing fatty acids which will provide glucose precursors as

well as usable energy during their oxidation will protect an animal from fasting hypoglycemia. Feeding the C_{17} diet, however, did not preserve liver glycogen. Glycogen was 1.4 percent of liver weight in the fed animal, whereas the liver of the fasted animals was depleted of all glycogen (Table IV).

Whether or not all changes observed in the C_{17} study are less severe because odd carbon chain fatty acids were present in the fat stores must wait on the results of our more complete study.

SIGNIFICANCE OF THIS STUDY

Our findings that diet can affect body composition and enzymatic activity make dietary history a factor to be considered in subjects likely to experience metabolic stress. The results of Feller and co-workers (17, 18) and of Smith's group (19) would indicate that abnormal gravitational forces produce such stress. Our study of the fasting state provides an opportunity to determine the importance of dietary history in the ability to withstand a stress. It will also provide information for selecting dietary conditions necessary to minimize nutritional stress. If dietary history does appear to be a significant factor, and at this stage it does, clearly it would be valuable to carry out similar studies in animals undergoing the stress of chronic acceleration and of weightlessness.

TABLE I

COMPOSITION OF ISOCALORIC DIETS

Component	Percent of Weight				Percent of Calories			
	S	P	M	C_{17}	S	P	M	C_{17}
Casein	22	60	28	28	23	65	22	22
Glucose	72	20	40	40	77	22	31	31
Hydrogenated Fat[1]	0	5	25	25	0	13	47	47
Salt Mix[2]	4	4	4	4	0	0	0	0
Vitamin Mix[3]	0.2	0.2	0.2	0.2	0	0	0	0
Celluflour	1.8	10.8	1.8	1.8	0	0	0	0

1. Diets M and P: Plastine, Durkee Famous Foods: Hydrogenated coconut oil. Fatty acid composition: C 8:0, 6.1%; C 10:0, 5.5%; C 12:0, 48.9%; C 14:0, 18.7%; C 16:0, 9.0%; C 18:0, 9.6%; C 18:1, 2.1%. Diet C_{17}: Heptadecanoic acid, Eastman Chemical.

2. Contained the following in grams: $CaCO_3$, 72.5; $CaHPO_4$, 113.0; Na_2HPO_4, 65.1; KCl, 40.0; $MgSO_4$, 23.0; $MnSO_4 \cdot H_2O$, 1.54; $CuSO_4$, 0.13; ferric citrate, 1.51; $ZnCO_3$, 0.21; and KIO_4, 0.01.

3. Contained the following in grams: choline bitartrate, 13.5; vitamin A palmitate (250,000 IU/gm), 0.08; vitamin D_2 (500,000 IU/gm), 0.03; D-α-tocopherol acid succinate (890 IU/gm), 0.675; menadione sodium bisulfite, 0.002; thiamine hydrochloride, 0.0125; riboflavin, 0.025; pyridoxine hydrochloride, 0.012; niacinamide, 0.15; calcium pantothenate, 0.08; vitamin D_{12} (0.1% in gelatin), 0.005; folic acid, 0.005.

TABLE II

METABOLIC ACTIVITY OF LIVER AND ADIPOSE TISSUE BEFORE AND AFTER A 7-DAY FAST OF RATS FED A DIET CONTAINING HEPTADECANOIC ACID AS THE SOLE SOURCE OF FAT

		Fed	Fasted
Enzymatic activity: (μm/gm Protein/min)	In liver:		
	Hexokinase	27.4	12.9
	Glucose-6-P dehydrogenase	24.8	27.8
	6-P-gluconate dehydrogenase	16.8	18.9
	Malic enzyme	19.0	4.3
	Citrate lyase	4.3	0.9
	Pyruvic carboxylase	48.8	44.0
	Pyruvic carboxykinase	9.2	19.8
	In adipose tissue:		
	Hexokinase	44.4	43.8
	Glucose-6-P dehydrogenase	2.2	24.0
	6-P-gluconate dehydrogenase	4.4	9.2
	Malic enzyme	22.2	2.5
	Citrate lyase	1.8	2.2
Biosynthetic capacity[1]: (μm/gm Tissue/3 hrs)	In liver:		
	CO_2 from alanine	26.4	55.8
	Glucose from alanine	3.2	7.0
	Fatty acid from acetate	1.2	0.12
	In adipose tissue:		
	CO_2 from acetate	1.5	22.1
	Fatty acid from acetate	0.2	2.8

1. Incorporation of tracer substrate.

TABLE III

FATTY ACID COMPOSITION OF LIVER, ADIPOSE TISSUE AND CARCASS BEFORE AND AFTER A 7-DAY FAST OF RATS FED A DIET CONTAINING

Tissue	Treatment	Solids (% wet wt.)	Fatty Acids (%[2])	Fatty Acids[1], % Total Fatty Acids									
				12:0	14:0	14:1	16:0	16:1	17:0	18:0	18:1	18:2	20:4
Carcass	Fed		7.67	0.1	1.7	0.3	19.9	4.9	3.1	4.7	30.5	27.3	2.3
	Fasted		.91	0.1	0.6	0.2	18.6	3.0	4.4	16.4	22.3	13.1	7.0
Fat Pad	Fed	88.87	78.95	0.1	1.6	0.3	16.6	4.9	1.0	3.2	32.9	31.7	2.1
	Fasted	21.54	2.60	0.1	1.1	0.1	46.2	1.8	3.5	22.3	34.4	12.9	25.3
Liver	Fed	30.19	2.47	0.1	0.3	-	10.7	1.3	10.5	8.0	13.2	20.7	30.0
	Fasted	28.18	1.46	-	0.3	-	11.8	1.0	4.3	20.1	12.0	13.5	28.8

1. Designated as number of C atoms in chain followed by the number of C=C.

2. Carcass fatty acids expressed as percent of wet weight. Tissues are expressed as percent of total solids.

TABLE IV

CONCENTRATION OF PLASMA GLUCOSE AND FREE FATTY ACIDS AND OF HEPATIC GLYCOGEN BEFORE AND AFTER A 7-DAY FAST FROM RATS FED A DIET CONTAINING HEPTADECANOIC ACID AS THE SOLE SOURCE OF FAT

Treatment	Concentration in Plasma of:		Hepatic Glycogen
	Glucose	Fatty Acids	
	(mg/100 ml)	(μEq/L)	(%)
Fed	140	1009	1.4
Fasted	114	1105	0

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